

BIOLOGICAL BULLETIN

THE PRODUCTION OF FUNCTIONAL AND RUDIMENTARY SPERMATOOZOA IN ROTIFERS.¹

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Marked dimorphism in the spermatozoa of a few animals has been briefly recorded by several observers, but no detailed study has been made of such forms to determine how the two kinds of spermatozoa are formed nor has there been offered an explanation as to their probable functions. While looking at the plates in "The Rotifera," by Hudson and Goss, I noticed sketches of the two dimorphic spermatozoa of the marine rotifer, *Brachionus mulleri*. As this rotifer occurs in countless numbers in a salt-water lake near Lincoln, Neb., an excellent opportunity was offered to reëxamine it in an attempt to find an explanation for the production of the two kinds of spermatozoa. After reëxamining the spermatozoa of this rotifer and confirming the observations of Hudson and Goss the spermatozoa of eight other species of rotifers were examined and were found also to be composed of two kinds. Thus indicating that this phenomenon of dimorphism of spermatozoa may be general for all the species of the whole group of rotifers which produce male individuals.

The technique of examining the spermatozoa is very simple. Mature free swimming males were placed on a slide in a drop of the culture water and covered with a cover glass. Then by pressure the males were forced to eject the contents of the single testis into the water. The two kinds of spermatozoa could be readily seen even with the low powers of the microscope and were as easily counted. Early stages in the development of the spermatozoa as far back as the early spermatids were studied by crushing the eggs containing the unhatched immature males. All stages of development from the earliest spermatid up through

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to a mature spermatozoön were easily seen, especially in the species of rotifers whose males form only a few spermatozoa.

Brachionus mulleri, the salt-water rotifer, was the first species examined and because of the relatively small number of spermatozoa produced by the males it proved to be the most favorable form in which to see the early stages in the development of the spermatozoa as well as for making accurate counts. The motile spermatozoa were extraordinarily large and measured about one third the total length of the male individual that produced them. Probably on account of the large size of the spermatozoa a male is physically unable to form very many. These motile spermatozoa were long and somewhat slender with a pointed anterior end. The posterior two-thirds of the spermatozoön is very motile and has a prominent, undulating, median, and dorsal membrane which aids greatly in the locomotion. The sketch of a mature spermatozoön in Fig. 1, *E*, is a dorsal view and does not show adequately this membrane. These spermatozoa swim freely about in the water for a short time after they are forced out from the body of the male.

The spindle-shaped spermatozoa are incapable of any locomotion. They are uniform in shape and never change their form. They are very much smaller than the motile spermatozoa. Fig. 2, *D*, shows several of these. As the tissues of the males are very transparent both kinds of these spermatozoa may be readily seen inside the testis. The spindle-shaped spermatozoa are usually found collected together near the posterior end of the testis, although sometimes only a few are thus collected together while the remainder are scattered throughout the testis among the motile spermatozoa.

When the immature males are crushed the spermatids still united in masses separate readily from the general debris. Various stages in the development of the spermatids and spermatozoa may be seen in accordance with the stage of development of the male. All spermatids or immature spermatozoa in each male are generally found in the same stage of development.

The relative number of the two kinds of spermatozoa varied widely when counts were made from the mature free swimming males taken from a general culture of rotifers. It is very prob-

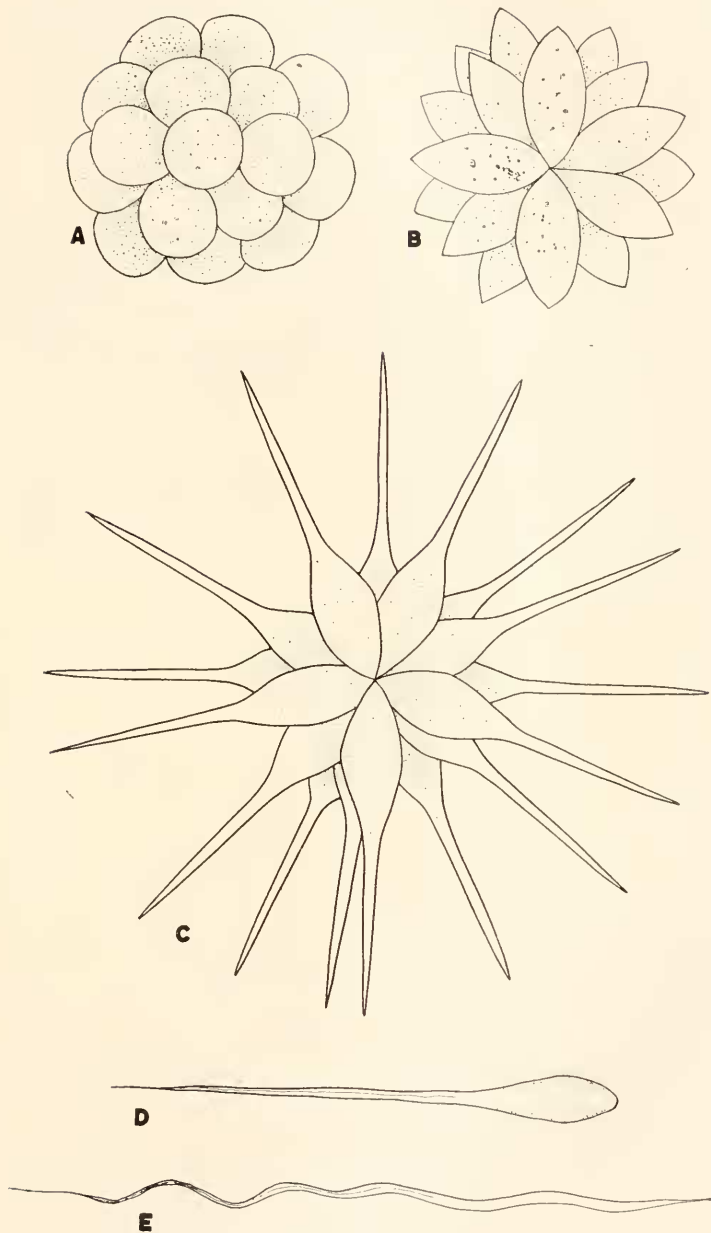


FIG. 1. Formation of the functional spermatozoa in *Brachionus mulleri*.
 A, young spermatids; B, older spermatids; C, young immature spermatozoa;
 D, older immature spermatozoön; E, mature, motile, and functional spermatozoön.

able that many of these males had already paired with females and consequently had shed some of their spermatozoa. This would explain the variability of the spermatozoa counts in the first twenty-one males recorded in Table I.

TABLE I.

Brachionus mulleri. Showing the relative proportional number of the two kinds of spermatozoa found in each testis of thirty male individuals. The rudimentary spermatozoa were never found as numerous as the functional spermatozoa in any testis of a young male. In the immature males in which none of the spermatozoa had been fully formed or shed the larger spermatids that ultimately develop into the functional spermatozoa were found to be exactly twice as numerous as the smaller spermatids that ultimately develop into the rudimentary spermatozoa.

Serial Number.	State of Development of Males.	Number of Functional Spermatids or Spermatozoa in Testis.	Number of Rudimentary Spermatids or Spermatozoa in Testis.	Serial Number.	State of Development of Males.	Number of Functional Spermatids or Spermatozoa in Testis.	Number of Rudimentary Spermatids or Spermatozoa in Testis.
1	Mature	50	26	16	Mature	5	0
2	"	48	—	17	"	24	8
3	"	—	12	18	"	9	10
4	"	—	0	19	"	28	10
5	"	6	2	20	"	18	6
6	"	—	15	21	"	60	25
7	"	2	1	22	Immature	38	18
8	"	20	11	23	"	70-80	38-40
9	"	—	18	24	"	40	18-20
10	"	22	13	25	"	40	18-20
11	"	4	0	26	"	60	32
12	"	15	5	27	"	64	32
13	"	56	35	28	"	40	20
14	"	28	11	29	"	56	28
15	"	40	22	30	"	48	24

When immature males were crushed one could readily count all the spermatids of both kinds and could definitely determine the relative number of each. It was seen at once when all spermatids were counted that the two kinds of spermatids that develop into the two kinds of spermatozoa did not occur in equal numbers. The large spermatids which ultimately develop into the large motile spermatozoa were found to be exactly twice as numerous as the smaller spermatids which ultimately develop into the small spindle-shaped spermatozoa. This is clearly shown in the last few males recorded in Table I. Figs. 1 and 2

show a series of the various stages of development from early spermatids to mature spermatozoa of both kinds of these dimorphic spermatozoa.

Mature males of *Hydatina senta* were crushed and examined and were found also to possess the two kinds of spermatozoa as markedly dimorphic as those in *Brachionus mulleri*. Many more spermatozoa are formed in the testis and consequently the counts could not be as readily or as accurately made as in *Brachionus mulleri*. Counts enough, however, were made to show that the large motile spermatozoa were about twice as numerous as the smaller immotile spindle-shaped spermatozoa.

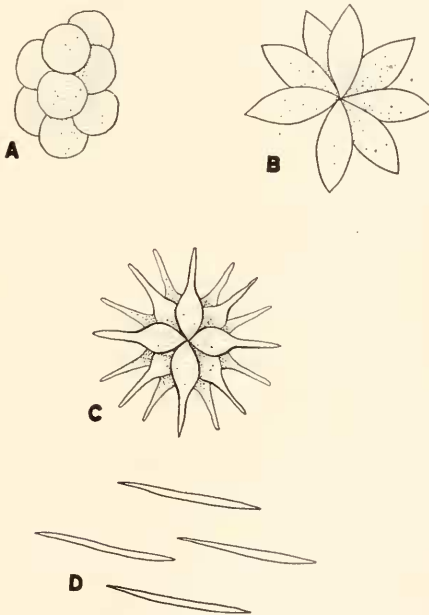


FIG. 2. Formation of the rudimentary spermatozoa in *Brachionus mulleri*. A, young spermatids; B, older spermatids; C, young immature spermatozoa; D, mature, immotile, and rudimentary spermatozoa.

The counts are shown in Table II. and drawings of the two kinds of spermatozoa are shown in Fig. 5.

The spermatozoa were examined next of the large viviparous rotifer, *Asplanchna amphora*, and although no very accurate counts could be made the two kinds of spermatozoa were con-

spicuously present. The highest count for the motile spermatozoa was about 200, while the highest count of the immotile spindle-shaped spermatozoa was about 100. These counts were made from males which were isolated at birth before they

TABLE II.

Hydatina senta. Showing the relative proportional number of the mature dimorphic spermatozoa found in each testis. In general the large motile spermatozoa were twice as numerous as the small spindle-shaped spermatozoa.

Serial No.	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.	Serial No.	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.
1	Mature	60	18	8	Mature	—	50-60
2	"	—	24	9	"	50-60	25-30
3	"	72	26	10	"	0	5
4	"	160-200	—	11	"	20	18
5	"	130	44	12	"	186	—
6	"	—	40	13	"	90	52
7	"	—	30				

had had an opportunity to pair with females and to shed their spermatozoa. They show that the motile spermatozoa are about twice as numerous as the immotile kind. Drawings and counts of these two kinds of spermatozoa are shown in Fig. 3 and Table III.

TABLE III.

Asplanchna amphora. Showing the relative proportional number of the mature dimorphic spermatozoa found in each testis. In general the large motile spermatozoa were twice as numerous as the small spindle-shaped spermatozoa.

Serial Number	State of Development of Males.	Number of Functional Spermatozoa in Testis.	Number of Rudimentary Spermatozoa in Testis.
1	Mature	200	50
2	"	200	60
3	"	—	104
4	"	160	75

In addition to the spermatozoa of these three species of rotifers already described the spermatozoa of six other species of rotifers were carefully examined. These species were *Polyarthra platyptera*, *Diglena catellina*, *Euchlanis dilatata*, *Metopidia lepadella*, *Brachionus urceolaris* and *Brachionus bakeri*. In all cases the

two kinds of spermatozoa were found and the larger motile ones were more numerous than the smaller immotile ones. Drawings of these spermatozoa are shown in Figs. 4, 6, 7, 8, 9 and 10.

Morgan has found in the study of the spermatogenesis of some of the phylloxerans that the secondary spermatocytes are of two kinds—the normal and the rudimentary. The normal spermatocytes divide again to form the spermatids, each of which develops later into a functional spermatozoön. The rudimentary spermatocytes do not divide again and do not form spermatids or spermatozoa. They merely persist for a time as rudimentary secondary spermatocytes and then finally disintegrate and disappear. Morgan has also found the same phenomenon in some of the aphids, Stevens and von Baehr have also found it in the aphids, Meves has found it in *Vespa germanica*, Mark and Copeland have found it in *Vespa maculata*, and Lams has found it in an ant, *Camponotus herculeanus*.

In the grand total count of all the spermatocytes and spermatids at the end of the final spermatocyte divisions in all of these forms enumerated in the preceding paragraph the normal spermatids should have been twice as numerous as the rudimentary spermatocytes. No counts, however, were made and consequently this point was not settled by this method but it was demonstrated in another manner by showing that only one spermatocyte division occurred in the rudimentary spermatocytes and two divisions occurred in the normal spermatocytes in the process of formation of the normal spermatozoa. In 1909 Morgan said in regard to the rotifer, *Hydatina senta*, which at the time had not been investigated. "Theoretically there should not be two spermatocyte divisions but only one true division. It remains to be seen whether this prediction proves true."

The rotifers have not only fulfilled but have exceeded Morgan's expectations! They not only produce normal spermatids and rudimentary secondary spermatocytes in the ratio of 2:1 as probably do these forms already mentioned, but they develop both of these into spermatozoa which are later shed by the males. Those of one kind are large, motile and probably functional while those of

3. *ASPLANCHNA*
AMPHORA4. *POLYARTHRA*
PLATYPTERA5. *HYDATINA*
SENTA6. *DIGLENA*
CATELLINA7. *EUCHLANIS*
DILATATA8. *METOPIDIA*
LEPADELLA9. *BRACHIONUS*
URCEOLARIS10. *BRACHIONUS*
BAKERI

FIGS. 3-10. Dimorphic spermatozoa of eight species of rotifers showing the larger, motile, and functional spermatozoa and the smaller, immotile, and rudimentary spermatozoa. In all of these species the drawings were made of spermatozoa that were taken from mature free-swimming males except in Fig. 4 which were made of spermatozoa of unhatched immature males. All were drawn to same scale as Figs. 1-2.

the other kind are small, immotile and probably rudimentary, and without function. The rudimentary spermatozoa must necessarily develop directly from the secondary spermatocytes without the occurrence of the second spermatocyte division in order to form the ratio of two normal spermatozoa to one rudimentary spermatozoön. If they developed from rudimentary spermatids which were formed by the division of the secondary spermatocytes the ratio would be one functional spermatozoön to one rudimentary spermatozoön. This interpretation of the observed ratios would therefore place the rotifers in the same category as the other animals already mentioned as far as the formation of normal spermatids and of rudimentary secondary spermatocytes are concerned. They would, however, have this point of difference, viz.: that the degree of degeneration of these rudimentary secondary spermatocytes has not progressed as far as to render such spermatocytes incapable of developing directly into degenerate spermatids and spermatozoa of a certain kind, as is the case of the other forms studied.

In the mature parthenogenetic egg that develops into the male individual there is the reduced number of chromosomes. This contains one half as many chromosomes as are found in the parthenogenetic egg that develops¹ into the female. This fact was made out in *Hydatina senta* but the exact number of chromosomes occurring in each kind of egg has never been determined. If the male in the beginning has already the reduced number of chromosomes no further reduction of chromosomes is necessary in the formation of the spermatozoa. The race, however, may have retained the former ancestral process of performing the first spermatocyte division, regardless of the results, in the formation of the secondary spermatocytes but as there is no general reduction of chromosomes to be made at this stage presumably none occurs. One half of the secondary spermatocytes very likely contain fewer or perhaps no chromosomes at all and consequently are very degenerate and rudimentary and without a second division develop directly into degenerate spermatids and spermatozoa.

The male parthenogenetic eggs are the ones that are fertilized as has been determined by Maupas, Lauterborn, Whitney, and

Shull and after fertilization always develop into females but never into males. Thus the fertilization causes a change of the sex in the resulting individual from such eggs. All of the functional spermatozoa must therefore be identical in their influence in producing females from eggs that if unfertilized would otherwise have produced males.

SUMMARY.

1. Dimorphic spermatozoa are produced and shed by the rotifers, *Brachionus mulleri*, *Asplanchna amphora*, *Polyarthra platyptera*, *Hydatina senta*, *Diglena catellina*, *Euchlanis dilatata*, *Metopidia lepadella*, *Brachionus urceolaris* and *Brachionus bakeri*.

2. One class of the spermatozoa is composed of large, vermiform, and motile spermatozoa and the other class is composed of small, spindle-shaped and immotile spermatozoa.

3. The number of motile spermatozoa produced is exactly twice the number of immotile spermatozoa produced.

4. The motile spermatozoa probably develop from the cells formed by the second division of the normal spermatocytes but the immotile spermatozoa probably develop directly from the rudimentary cells formed by the first division of the spermatocytes.

5. The motile spermatozoa are probably functional but the immotile spermatozoa are probably rudimentary and functionless.

6. The functional spermatozoa are all identical in their power of determining the sex of the individual that develops from a fertilized egg. After a functional spermatozoön has fertilized a parthenogenetic male egg the egg eventually develops into a female individual instead of developing into a male individual as it otherwise would have done if it had not been fertilized.

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November 3.—In some recent and more careful studies on the spermatogenesis of *Brachionus mulleri* it has been determined that the bodies which have been designated as normal spermatozoa are only the detached tails of these spermatozoa and are not the complete cell. When the males are compressed under a cover glass the tails become detached from the heads of the spermatozoa while inside the body of the males and then are immediately extruded and remain in an active state for a considerable length of time. This fact was not observed by Hudson and Goss as is shown by their plate 27 nor by myself until very recently.

As these motile tails resemble very closely the motile spermatozoa of the other species of rotifers described in this paper it is very probable that all the motile and supposedly complete spermatozoa observed were also only tails. Whether the bodies that were considered rudimentary spermatozoa are only tails of such spermatozoa or are complete spermatozoa has not been definitely decided.

These new detailed facts do not, in the least manner, invalidate the main issue of the paper in regard to the two classes of spermatozoa and their ratio. Additional observations and data derived from these recent studies will be published shortly.

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